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GUIDANCE FOR THE DERIVATION OF AN ACUTE REFERENCE DOSE

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The Inter-Organisation Programme for the Sound Management of Chemicals (IOMC) was established in 1995 following recommendations made by the 1992 UN Conference on Environment and Development to strengthen co-operation and increase international co-ordination in the field of chemical safety. The Participating Organisations are FAO, ILO, UNEP, UNIDO, UNITAR, WHO and OECD. The World Bank and UNDP are observers. The purpose of the IOMC is to promote co-ordination of the policies and activities pursued by the Participating Organisations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

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FOREWORD

The objective of this document is to provide guidance on how to derive an Acute Reference Dose (ARfD). It outlines a stepwise approach on how to best use all available toxicological data, and what to do if more data are needed on the refinement of the toxicological data base with regard to the acute effects of the compound being investigated or an advanced exposure assessment, if the risk assessment indicates a human health concern.

It is not intended that this guidance provide a new Test Guideline or to encourage additional animal testing. The results of a recent retrospective analysis confirmed that the development of a design for an acute study that produces more comprehensive toxicological data for setting ARfDs would be valuable but that such a special ARfD study would become necessary for very few pesticides only. Therefore, guidance on the design and performance of a single dose test is provided on the basis that if a single exposure study is necessary, it should be performed according to a harmonised OECD test procedure.

This guidance document is primarily intended for pesticides, biocides and veterinary drugs, but could be used for all categories of chemical substances which may be ingested by human beings in food and/or drinking water as well as non-dietary oral exposure. The general principles and concepts can also be applied to dermal and inhalation exposure routes. However, these routes would more appropriately be addressed in separate OECD guidance documents. An OECD Guidance Document is under development for setting acute reference concentrations for the inhalation exposure route.

The proposal to develop this guidance document was submitted by Germany in 2007. The Working Group of National Coordinators of the Test Guidelines Programme (WNT) was requested to comment on successive drafts in November 2007, February 2009, and November 2009. The draft document was also submitted to the Working Group on Pesticides for comments. A small drafting group meeting was held in Germany in October 2008, and an expert group meeting was hosted in Geneva in September 2009, hosted by the WHO.

The WNT slightly revised the draft guidance document and approved the revised draft guidance document at its meeting held on 23-25 March 2010. The Joint Meeting of Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology agreed to its declassification on 1 June 2010.

This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology.

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Background

1. Regulatory requirements or legislation relating to the protection of human health have led to the establishment of Acute Reference Values (ARVs) for substances which would cause toxic effect after acute human exposure. This approach applies primarily to pesticide, biocide, and veterinary drug residues in food and drinking water for which Acute Reference Doses (ARfDs) have to be considered (1) (2) (3) (4) (5). Regulatory authorities are required to protect the general population including susceptible groups against effects induced by acute oral exposure to hazardous substances, if the Tolerable/Acceptable Daily Intake (TDI/ADI) is likely to be substantially exceeded for short periods of time (6) (7).

2. The ARfD of a chemical is an estimate of the amount of a substance in food and/or drinking water, normally expressed on a body weight basis that can be ingested in a period of 24 hours or less, without appreciable health risk to consumer, on the basis of all the known facts at the time of the evaluation (2).

3. Various guidance documents are available for setting ARVs (1) (2) (3) (4) (7) (8). The WHO panel of the Joint Meeting on Pesticide Residues (JMPR) adopted general considerations in setting of ARfDs for pesticide chemicals (1) (2). Solecki et al. (1) described in detail a stepwise process for establishing ARfDs, as well as specific considerations and guidance regarding the identification of the most appropriate critical effects for selected toxicological endpoints. The general principles and methods for setting an ARfD for chemicals in food have been recently updated by IPCS (9) (10).

4. For a critical effect, a no-observed-adverse-effect-level (NOAEL) that is typically determined from animal studies has been traditionally used as a Point of Departure (PoD) when deriving an ARfD. An alternative method to derive a PoD is the use of the Benchmark Dose (BMD) modelling approach response (11). The BMD is defined as the dose producing a predetermined level of change in response (such as a 10% increase in the incidence of a particular toxic effect) compared with the background. A BMD is derived by fitting a mathematical model to the dose-response data, and is often accompanied by an estimate of the statistical lower confidence limit (BMDL) on the BMD.

5. The JMPR ARfD guidance acknowledged that endpoints from a repeat dose toxicity study could be used for setting an ARfD if the critical effect of the compound has not been adequately evaluated in a single exposure study. The JMPR recognised that this approach is likely to be conservative.

6. A retrospective analysis of ARfD values was conducted on 198 pesticides, which have been evaluated and peer-reviewed in Europe and were included in Annex I of EU directive 91/414/EEC in the time period between the years 2000 and 2008 (9) (12). For 48 % of all substances, no ARfD was considered necessary because of the low acute toxicity of these pesticides. The majority of ARfDs was based on studies which are regularly required for pesticides and in which specific acute alerts were investigated. In less than 10 % of cases, conservatively established ARfD values were based on repeated dose toxicity or multigeneration studies. For 4 of these pesticides (i.e. 2 %) a refinement of the ARfDs using an additional toxicity study would be justified because refinement on the exposure side was not sufficient. In the analysed database, such special studies for ARfD refinement were submitted for 4 % of the 198 pesticides. They were mostly performed in addition to the basic acute toxicity data requirements, if it was apparent that the acute intake estimation exceeded a conservatively established ARfD. However, in some cases such studies were not accepted by the authorities because of quality deficiencies.

7. The results of this recent analysis confirmed that the development of a harmonised acute study design that produces more comprehensive toxicological data for setting ARfDs would be valuable.

8. In a few cases, the results from monitoring programs may provide residue data showing that a conservatively derived ARfD is exceeded. To estimate if there is a real human health risk, a refinement of the risk assessment might be performed first on the basis of exposure calculation and second on the basis of a PoD from a more appropriate special single exposure study. Such a study could be undertaken as a last resort to perform a more realistic human health risk assessment or to justify the lowering of Maximum Residue Levels (MRLs) or the withdrawal of an authorisation of a plant protection product.

9. The ILSI Health and Environmental Sciences Institute (HESI), through its Agricultural Chemical Safety Assessment (ACSA) Committee, designed a toxicity testing scheme for agricultural chemicals that uses a tiered approach. It moves away from paradigms that involve extensive animal testing for 'every possible adverse outcome' to an approach which focuses more on the needs of the risk assessment (13). The HESI ACSA Task Force devised a set of studies in a tiered approach which could provide information for the most relevant human exposure periods and outlined a draft protocol for a single exposure test in dogs or rodents as an optional step five (14). Doe et al. (14) proposed that the ARfD could be based on 28-day rat and 90-day dog studies with additional parameters and if the ARfD derived from the repeat dose study indicates an adequate margin of exposure, then the performance of a single exposure study would not be necessary. The authors emphasized that if a single exposure study is considered necessary, existing data/knowledge should be considered to determine the relevant endpoints and the most appropriate species (rat or dog).

10. The tiered approach proposed by HESI ACSA to determine the need for a single exposure study is consistent with the stepwise approach outlined by Solecki et al. (1). As emphasized by Solecki et al. (1) and by Doe et al. (14), results of existing toxicity data, combined with knowledge of potential human exposure, should be used to determine the need for an acute exposure study. The available toxicity studies may also guide whether the dog or the rat should be used.

11. The single exposure study design proposed by the JMPR (2) and Solecki et al. (1) and adopted by the HESI ACSA Task Force (14) forms the basis of the guidance on how to perform and tailor a single exposure test (Annex 2).

12. The results of the single exposure study should (i) clarify whether a substance poses an unacceptable acute risk and (ii) allow the derivation of a refined ARfD for acute intake of residues in food and/or drinking water.

13. At the 13th Meeting of the OECD Working Group on Pesticides in 2002, the JMPR presented a proposal for an OECD Test Guideline animal study, designed to support the JMPR-Guidance to derive an ARfD for dietary risk assessment for human health (15). The EC, Spain, and Crop Life International supported this proposal, suggested improvements and recommended that JMPR should proceed by approaching the Working Group of National Co-ordinators to the Test Guidelines Programme (WNT).

14. The 19th WNT meeting then agreed that an improved stepwise approach for a harmonised guidance document, but not an OECD Test Guideline animal study, should be developed for the derivation of an ARfD. The WNT agreed to include the project in the work plan for 2007 (16).

Purpose

15. The objective of this document is to provide a stepwise approach for a harmonized guidance on how to set an ARfD based on all appropriate existing toxicological and exposure data. The general considerations in the setting of an ARfD in an enhanced stepwise process, as well as specific considerations and guidance regarding the identification of the most appropriate critical effects for selected toxicological endpoints are described. The general biological background and the data available through standard toxicological testing for regulatory purposes, interpretation of the data,

conclusions and recommendations for future improvements are described for these selected relevant endpoints. Special emphasis is placed on evaluating whether toxic effects observed in the standard package of repeat dose toxicity studies may also occur after single doses.

16. Data are not often available for many types of effects under acute exposure conditions and it is possible that the PoDs and endpoints that will be critical for setting an ARfD may differ from those for setting chronic RfDs, or ADIs. The general principle is agreed that the ARfD should be equal to or greater than other long-term reference values of the same chemical (i.e. an individual can generally tolerate a higher amount of a substance with an acute exposure than with a repeat exposure). This is important because ARfDs are typically coupled with high-end exposure values rather than the average exposure values that are employed in risk assessments involving repeat exposures.

17. This guidance document is intended to promote a harmonised scientific basis for the derivation of ARfDs suitable for refined acute risk assessment in a range of acute human oral exposure scenarios.

18. This proposed guidance document will

- Replace the need to conduct unnecessary tests on animals by introducing a tiered approach for human health risk assessment, including a refined exposure assessment,
- Reduce the need to repeat animal tests which have not been performed in a way which adequately satisfy the requirements of different regulatory agencies, and
- Refine a harmonised procedure for determining an ARfD of a compound in infrequent exposure situations where it is necessary to adequately characterise the acute hazard.

19. This document presents specific guidance on

- How to select relevant endpoints and PoDs from the existing database,
- How to refine the exposure calculation for the acute risk assessment in Annex 1, and
- How to perform a tailored single exposure study (Annex 2), including the minimum parameters that should be examined depending on all existing data to allow the derivation of a PoD for the most relevant acute effect(s) in the most appropriate species; however, this is not intended to become a routine data requirement.

Basic Considerations

20. The derivation of an ARfD should be done based on following the tiered approach which is specified in more detail in Section D.

21. The appropriateness of all available endpoints from all existing oral toxicity studies to establish ARfDs needs to be carefully considered as a ***first step***. The pertinent biology of the system affected should be considered to determine whether an acute exposure may compromise the ability of the organ to compensate and maintain homeostasis. Particular weight should be given to observations and investigations at the beginning of repeat dose studies. However, this approach should also consider such acute effects involving C_{max}-dependent and rapidly reversible effects to ensure that acute effects from repeat dose dietary studies are not underestimated. Isolated findings, showing no specificity or clear pattern are not necessarily indications of toxicity. In the absence of information to the contrary, all toxic effects seen in repeat dose studies should be evaluated for their relevance in establishing an ARfD.

22. For determination of the PoD, the most sensitive endpoint in the most sensitive species must be selected with appropriate toxicological expertise to avoid selecting an endpoint in a given species particularly sensitive to that endpoint under normal conditions.

23. After reviewing the available toxicological database, the possible exposure scenarios should be considered as a ***second step***, based on the guidance in Annex 1. A tiered human health risk assessment should be conducted that includes a comparison of the ARfD with the potential acute oral exposure (or internal body burden), based on a worst-case assumption. If this worst-case assessment does not indicate unacceptable health risks, no further refinement of the acute risk assessment may be warranted. However, if this risk assessment indicates a borderline or a clear concern, then the next tier should focus on a further refinement of the exposure assessment (from refined acute intake estimation). Models used for the calculation of dietary exposure are based on the premise that intake is a function of the concentration of pesticide in food and the amount of food consumed (9). For that reason a more elaborated analysis of the actual acute dietary exposure as well as toxicological assessments do allow for a more realistic approach for determining the actual risk in quite a number of cases. If the health risks are now acceptable, no further refinement is warranted.

24. If the refined exposure assessment still shows unacceptable health risks and if conservative assumptions were used in setting the ARfD, then as a ***third step*** consideration may be given to conducting a single exposure study to establish a refined ARfD and how to perform and tailor this single exposure test, based on the guidance in Annex 2. This will only be necessary for a very limited number of substances according to the retrospective analysis (11).

Tiered-Approach for the Derivation of an appropriate ARfD

Step One

1. *Evaluate the total database of the substance, establish a toxicological profile for the relevant exposure periods to this substance and determine if there is an adverse effect occurring as a result of a single oral dose up to the limit dose.*

2. *Consider the principles for not setting an ARfD up to the limit dose*

25. The upper limit for a relevant ARfD was considered with reference to the potential range of dietary exposures to acutely toxic pesticides. The estimated maximum exposure of 1 mg/kg bw is conservatively based on a 50 kg person consuming in a single sitting a large food item (e.g., 500 g) treated with pesticide having a MRL of 20 mg/kg. A variability factor of 5 was applied. The cut-off of 500 mg/kg bw/day would allow the derivation of an ARfD of 5 mg/kg bw with an assessment factor of 100 and an additional Margin of Exposure (MoE) of 5.

- No findings indicative of adverse effects elicited by an acute exposure are seen at doses which are relevant for the acute risk assessment, i.e. up to about 500 mg/kg bw/day for residues of pesticides, justification see (1).

- However, establishment of ARfD should be considered if mortalities are observed at doses up to 1000 mg/kg bw in single oral exposure studies and the observed mortalities are relevant to human exposures.

26. If the above criteria do not preclude the setting of an ARfD up to the limit doses, then further consideration should be given to setting a value, using the most appropriate endpoint in the most relevant species.

3. *Selection of appropriate endpoints for setting an ARfD*

- Select the toxicological endpoints most relevant for a single (day) exposure in the most relevant species.
- Select the most relevant or adequate study in which these endpoints have been adequately determined.
- Identify the PoD for these endpoints.
- Select the most relevant endpoint providing the lowest PoD.

27. An endpoint from a repeat dose toxicity study should be used if the critical effect of the compound has not been adequately evaluated in a single exposure study and the effects in the repeat dose study are observed early on (e.g., early resorptions, etc). This is likely to be a more conservative approach and should be stated.

28. If after consideration of all the endpoints in appropriate available studies, an ARfD is not set, then the reasons should be justified and explained.

4. Selection of appropriate assessment factors for setting an ARfD.

29. The selection of appropriate assessment factors for inter-species and human inter-individual variability should be considered based on available data. To allow for the quantitative incorporation of specific information on toxicokinetic/toxicodynamic differences for a chemical, the IPCS recommended (17) (18) that the two 10X factors (for inter-species and inter-individual) each be divided into toxicokinetic/toxicodynamic sub factor for inter-species and human inter-individual differences as shown below, where available information on one or more specific sources of variability could be replaced.

30. A combined assessment factor may be based on (AKAF or AKUF) × (ADAF or ADUF) × (HKAF or HKUF) × (HDAF or HDUF)

- where AK represents inter-species toxicokinetic variability
- AD represents inter-species toxicodynamic variability
- HK represents human interindividual toxicokinetic variability
- HD represents human interindividual toxicodynamic variability
- AF represents a chemical-specific adjustment factor (CSAF)
- UF represents a default assessment sub-factor

31. In determining the appropriate assessment factor, a stepwise approach is proposed.

- Determine whether the database is adequate to support the derivation of a CSAF (17). IPCS recommended "default sub factors", i.e. 4-fold and 2.5-fold for inter-species toxicokinetic and toxicodynamic differences, respectively, and 3.16 for each of human interindividual toxicokinetic and toxicodynamic differences.

- Some reduction for human toxicokinetic differences from its default value of 3.16, may be justified. An example where the factor was reduced based on considerations relating to C_{max} and rate of elimination was recently documented by JMPR (18).

- If chemical specific toxicokinetic and toxicodynamic data are inadequate to justify data based assessment factors, consider if there is any information (e.g., QSAR, mode of action, on closely related compounds) that would indicate reduced or increased uncertainty.

- If no refinement is justified, the 100-fold (or 10-fold) default should be used. When using data obtained from animals, the default assessment factor is 100. This comprises a factor of 10 to allow for inter-species differences and a factor of 10 for intra-species (human inter-individual) differences. The overall assessment factor is the product of these two factors, i.e. 10×10.

32. Further to consideration of inter-species and human inter-individual variability, additional factors may be applied to address other uncertainties such as relating to completeness of the database and steepness of the dose-response curve, or to address an additional precaution in the case of especially severe effects.

33. Whenever an assessment factor other than a default is used, a clear explanation of the derivation of the factor should be provided. Individual countries may select assessment factors dependent on their specific regulatory requirements or national legislation.

Step Two (Annex 1)

5. *Application of the ARfD for the acute risk assessment*

- Determine whether the acute exposure estimate is exceeding the ARfD.

- If the acute intake estimation does not exceed the ARfD, no further refinement is necessary.

34. If the risk assessment indicates a borderline or a clear concern, then a refinement of the exposure assessment should be performed.

6. *Refinement of the exposure calculation for the acute risk assessment*

- In determining a refined exposure calculation, a stepwise approach is proposed.

35. If the risk assessment indicates still a clear concern, then a refinement of the ARfD could be performed.

Step Three (Annex 2)

7. *Experimental refinement of the ARfD derivation*

- As a last resort a single exposure study according to the test design in Annex 2 should be considered for the generation of data to establish and refine more appropriate ARfD.

Specific Guidance on the Derivation of ARfDs

36. Particular toxicology endpoints that are relevant to ARfD establishment are considered in the JMPR publication (2) and by Solecki et al. (1). Note that these documents are not intended to comprehensively cover all potentially relevant endpoints but focus on the interpretation of a number of selected endpoints which have proved to be problematic in reaching a decision as to whether an effect is relevant to an acute exposure.

Haematotoxicity

37. The induction of methaemoglobinaemia is considered to be a critical effect in consideration of acute responses to chemical exposure. For acute exposure to methaemoglobin-inducing xenobiotics, a level of 4% methaemoglobin (or higher) above background in dogs or a statistically-significant increase in rodents cf. background is considered to be relevant to set an ARfD (1). Haemolytic anaemias induced by mechanical damage, immune mediated anaemia, oxidative injury to RBCs and non-oxidative damage are considered to be less relevant for ARfD derivation since the severity of such effects appear to generally depend on prolonged exposure. If changes in haematological parameters are observed early in a repeat dose study and do not appear to progress during the course of the study, then such effects should be considered as relating to acute exposure to the substance. In assessing whether effects observed in repeat dose studies should be used for setting an ARfD, one should evaluate the mode of action. If known, this could provide arguments for selecting or not selecting the endpoint for setting an ARfD.

Immunotoxicity

38. Immunotoxicity data derived from currently available standard repeat exposure studies are not likely to be adequate for setting an ARfD due to lack of specific immunotoxic parameters and/or early observations. Even though effects on the immune system can be induced by a single exposure, typically a high dose may be required to cause immunotoxicity following a single exposure, except for chemicals that are eliminated slowly. Changes in immune function may be the result of overt toxicity (decreased food intake, irritation or inflammation, increased glucocorticoid release, or a general decline in fitness) rather than a direct effect on the immune system. Single dose effects on immune function are considered to be unlikely because the immune cells and mediators are constantly replaced and because of the inherent redundancy in the system (e.g., alternative mechanisms to resist infection). Potential concerns for acute effects would include auto-immune responses as well as effects on the immune system during development and hypersensitivity. As knowledge and methodologies evolve on immunotoxicity, this guidance may be reconsidered in the future.

Neurotoxicity

39. The nervous system has limited capacity for repair and regeneration. Therefore, any neurotoxicity seen in repeat dose studies could be the result of a single exposure that is not reparable, i.e. any evidence of neurotoxicity should be considered relevant to an ARfD assessment unless it can be demonstrated that the effects are produced only after repeat exposures. In addition to long-term or irreversible effects associated with acute exposure, attention should be paid to transient effects, as these could be considered as adverse under some circumstances.

40. Delayed neurotoxicity following single chemical exposures can occur and thus any acute exposure study should have an adequate period of investigation.

1. 41. In functional observation batteries (FOB) a large amount of data is produced; interpretation of such studies should include a consideration not only of the statistical significance of results but the nature, severity, persistence, dose-relationship and pattern of the effects. Isolated findings showing no specificity or clear pattern do not necessarily indicate neurotoxicity.

A common neurotoxic endpoint used to date in the derivation of ARfDs for insecticides is inhibition of acetylcholinesterase. The JMPR has previously defined criteria for the assessment of cholinesterase inhibition; these apply equally to the setting of ADIs and ARfDs.

Kidney and liver effects

42. If effects on these organs cannot be discounted as being either adaptive or as the result of prolonged exposure, an ARfD can be derived on the basis of these effects. Such an ARfD is likely to be conservative and it may be possible to subsequently refine it using an appropriately designed single exposure study. When interpreting data on liver and kidney toxicity in repeat dose studies, one should consider two important aspects, firstly, the type of effect observed and secondly, any information on correlations between exposure duration and effect.

43. For liver toxicity it is considered that findings of increased serum cholesterol, cirrhosis, induced activity of metabolising enzymes, regenerative hyperplasia, hepatocyte hypertrophy, fibrosis, or sclerosis in repeat dose studies are, in isolation, either adaptive or the result of prolonged exposure and therefore are not applicable for deriving an ARfD.

44. For kidney toxicity it is considered that the following findings of kidney toxicity in repeat dose studies are, in isolation, the result of prolonged exposure and are not applicable for deriving an ARfD: changes in organ weight; regenerative hyperplasia; altered serum calcium and phosphate.

Endocrine effects

45. In general, analysis should consider the mode of action of endocrine toxicity including human relevance, dynamics/kinetics, redundancy in the system, the ability of the organism to compensate and critical windows of sensitivity. If treatment- related adverse effects affecting development of the offspring, female reproduction function, or resulting in male germ Leydig or Sertoli cell toxicity were observed, it should be considered if

- These effects were caused by acute toxic key events directly related to interaction/disruption of hormonal system, and

- These effects are relevant for establishing ARfD,

e.g. the adverse effects are not considered to be secondary effects such as lesions via oxidative stress that cause deficit of hormone production/imbalance.

46. The nature of the effects, including their potential to occur following a single dose, as well as the dose-relationship and pattern of the findings should be considered in order to best ascertain whether endocrine findings are relevant for ARfD consideration. For example, an effect such as vacuolation in an endocrine tissue that is considered to be secondary to sustained alterations in steroidogenesis would be expected to be a consequence of repeated exposure, and thus not relevant as the basis of an ARfD. Further, as with any toxicological endpoint, isolated findings in endocrine tissues with no clear pattern or dose-response trend are not necessarily indications of toxicity. In most cases, thyroid hormonal effects in humans are unlikely to result from an acute exposure given the buffering capacity of the human thyroid system. It should be noted that endocrine toxicity is an evolving area and any guidance given is considered interim.

Developmental effects:

47. It is important to consider critical or sensitive windows of exposure that impact on the developing organism. Thus, any treatment-related adverse effect on fetuses or offspring which has resulted from exposure during any phase of development should be considered as potentially appropriate to use in acute dietary risk assessment, despite the fact that the treatment period typically consists of repeat dosing. Therefore, ARfD values that were derived from embryo/feto toxicity in rats or rabbits are considered appropriate to sufficiently protect women of childbearing age including the developing organism. But our knowledge on the mode of action of an acute exposure during a sensitive window

of fetal development and of the postnatal consequences of fetal observations is currently very limited. Furthermore, abnormalities of the maternal–fetal unit also need to be better described and taken into account for acute hazard characterization and risk assessment.

Maternal-toxic effects in developmental toxicity studies

48. ARfDs based on reductions in fetal bodyweight gain may be conservative and should be evaluated in the context of all pertinent data, including other developmental effects. Consideration should be given whether maternal toxicity is the result of repeat dose toxicity. If the maternal toxicity is due to an acute effect then maternal toxicity should be considered for setting an ARfD for the general population including women of child-bearing age. If the fetal body weight deficit occurs at a lower dose than the effect noted in the mother, the sensitivity of the young must be considered in setting a reference value.

Direct effects on Gastrointestinal (GI) tract:

49. Occasionally a chemical can cause adverse effects on the GI tract. These effects may be exerted through three different modes of action.

50. When GI effects occur, they are most commonly observed only after a bolus administration of a compound (by gavage or capsule) in fasted animals and administration of similar doses in food does not cause the same effects. In this case, the GI effects are most likely due to a local irritant effect of a high concentration of the compound in the GI tract. Since the ARfD applies to ingestion of a compound in food or drinking water, local GI effects exerted by bolus administration at very high doses, especially as hydrophobic solutions, may not be considered to be relevant for setting an ARfD.

51. Secondly, a chemical administered in food/diet may exert a local toxicological effect on the GI tract. Since the ARfD applies to chemicals in food, such an effect is likely to be relevant for setting an ARfD. For these direct effects, the modification of the inter- and intra-species toxicokinetic and toxicodynamic factors may be considered according to IPCS CSAF (17). However, such a change in the assessment factors should always be justified by explanatory text in the hazard and risk assessment document.

52. Thirdly, chemicals may exert an effect on the GI tract through a systemic action. For instance, it is known that the dopamine agonist apomorphine causes vomiting in humans and dogs, through a direct stimulation of the chemoreceptor trigger zone for emesis in the area postrema of the medulla oblongata of the CNS. Such an indirect effect on the GI tract is considered to be relevant for setting an ARfD. For such indirect GI effects, inter- and intra-species differences in the toxicokinetics of a substance should be taken into account according to (17) and (18).

53. The reasons for establishing (or not establishing) an ARfD on the basis of GI effects observed after single or short-term dosing, and the assessment factors applied should always be justified by appropriate explanatory text.

Other findings relevant for setting an ARfD

54. **Clinical signs** observed in acute oral toxicity studies (e.g., Median Lethal Dose (MLD) studies, acute neurotoxicty studies) or after one or several doses in repeat oral exposure toxicity studies may suggest the need to establish an ARfD.

55. Clinical observations can include.

- Respiratory observations (e.g., dyspnoea or laboured breathing, abdominal breathing, gasping and wheezing, apnoea or transient cessation of breathing, cyanosis, tachpnoea or quick and shallow breathing, colourless or red nostril discharge);

- Ocular signs (e.g., lacrimation, miosis, mydriasis, exophthalmos, ptosis, chromodacryorrhea, conjunctivitis, relaxation of nictitating membrane);

- Salivation, piloerection, changed muscle tone (generalised increase or decrease);

- Skin effects (e.g., redness of skin or erythema, oedema);

- Cardiovascular signs (e.g., change in heart rate, vasodilation or vasoconstriction, abnormal cardiac rhythm);

- Motor observations (e.g., changes in the level of spontaneous motor activity or locomotion, unusual locomotion, low body posture, preening, rearing, ataxia)

- Somnolence, anaesthesia, analgesia, catalepsy, prostration, tremors, muscle fasciculation; convulsions;

- Altered reflexes (e.g., external ear and corneal reflexes, loss-of-righting reflex);

- GI signs (e.g., vomiting, retching, changes in faecal output, including solid, dry or scant faeces, watery stools);

- Urinary effects (e.g., red urine, involuntary urination).

56. A more extensive discussion of clinical signs in animal studies can be found in OECD Guidance Document on the recognition, assessment, and use of clinical signs as humane endpoints for experimental animals used in safety evaluation (19), Chan & Hayes (20) or Derelanko (21). Note that some of these findings would not be easily detected by gross observation; for example, a standard MLD study. Rather, such findings would normally only be observed in more specific studies such as a tail-flick test for analgesia, a tail pinch test for anaesthesia or heart rate monitoring for cardiovascular effects. However, many of these clinical signs could be detected at gross observation by an experienced observer, leading to a consideration of the need conduct further detailed testing.

57. Changes in **bodyweight/bodyweight gain**, and **food and/or water intake** occurring in the observation period after acute dosing or in the first few days of repeat dose studies may be indicative of general toxicity, if it is clearly established that such effects are not based on palatability of the feed with which the test compound has been admixed. It is not uncommon for these parameters to be affected earlier in a study and at a lower dose than many other markers. A discussion of these endpoints may be found in OECD *Guidance Notes for Analysis and Evaluation of Repeat-Dose Toxicity Studies* (22). The effect of mixing the test compound in the diet needs to be considered. A compound administered in the diet may make the laboratory chow more or less palatable, or may possibly have a pharmacological stimulant or depressant effect on appetite. Likewise, decreased water consumption (e.g., in the case of an unpalatable compound administered in the water) will lead to reduced food consumption.

58. **Mortalities** in animals should be considered for setting an ARfD unless determined not to be relevant to human exposures. However, it would be very unlikely that mortalities would occur without observing clinical signs. According to OECD Test Guidelines, the highest dose level in toxicity studies generally should be chosen with the aim of inducing toxicity but not death or severe suffering.

59. Before deciding to choose death as an endpoint for setting an ARfD, every effort should be made to determine the cause or likely cause of death, including a careful examination of animals for clinical signs occurring at lower doses and/or with an earlier onset than death (19). The separation of deaths caused by factors unrelated to administration of the test agent (e.g., an acute or chronic infection, an anatomical abnormality, negligent handling or accident) from toxicity-induced deaths is important.

Animal Welfare Consideration

60. For reasons of animal welfare, the generation of additional animal data in a single exposure study should be justified in each particular case. It is recommended that such studies should NOT be performed in the situations below:

- If the derivation of an ARfD is considered unnecessary for toxicological reasons (e.g. no acute toxicity alerts) (1) (2),
- If acute toxicity studies enable an adequate evaluation of relevant effects.
- If repeat dose studies enable an adequate evaluation of critical acute effects observed early in the dosing period,
- If adequate developmental toxicity studies are available that indicate embryo/feto toxic effects in rats or rabbits are the most sensitive endpoints and it is NOT necessary to refine the ARfD for the general population,
- If a compound has very low residues in all relevant crops such that a refined dietary exposure estimates is not necessary and based on a sufficient margin of safety, no acute health risk for consumer is identified, or
- If based on the measured residues in all relevant crops the refined dietary exposure estimates indicate an adequate margin of safety even if measured against a conservative ARfD derived from a repeat dose study.

61. In the single exposure study, a minimum but sufficient number of animals of the most appropriate species should be utilised to produce the required additional data. Dogs should be used only when it has been demonstrated that they are the most sensitive species to the test substance if a single exposure study needs to be conducted.

62. If developmental toxicity in the rabbit provides the most sensitive endpoint, the critical PoD should be used for the ARfD derivation and no additional animal data are considered necessary. This guidance does not encourage additional studies in rabbits because there is normally no supporting information in the broader toxicology database and rabbits are prone to secondary effects due to stress (e.g., dosing and handling).

G. Consideration of Human Data

63. Individual countries have different regulatory environments regarding the use of human data. Therefore, appropriate use of human data is entirely dependent on the specific data and the regulatory situation in an OECD member country. Individual countries will select appropriate values dependent on their specific regulatory requirements or risk management policies.

64. Therefore, only considerations with regard to the scientific aspect of human information are given in this guidance. Human data may be available from accidental or deliberate poisonings, biomarker monitoring studies, epidemiology studies, volunteer studies. Human information on the same or structurally-similar compounds may provide useful data to help establish ARfDs.

65. The use of human volunteer data in chemical risk assessment is a controversial issue, with a range of views and specific regulatory requirements held by different OECD member countries. Therefore, the portion of ARfD values derived from human studies varies in a wide range among different authorities. In a retrospective analysis conducted in 2009 of EU ARfDs, only 0.5% (9) of the values were derived from human studies. In an older retrospective analysis not restricted to Europe approximately 10% of the ARfD values were derived from human studies (1). It is recognised that the use of human data may reduce the level of uncertainty inherent in extrapolating from animal models. For some substances like copper which is used as a pesticide but which is also an essential nutritional compound the results from human studies may be indispensable. There needs to be adequate consideration of both scientific and ethical issues. The JMPR has considered human data at many of its meetings. The JMPR reaffirmed the principle that endpoints from existing human volunteer studies could be very useful for setting health intake standards if the studies had been conducted in accordance with relevant ethical and scientific guidelines (2).

66. Due to the ethical implications of studies in humans, they should be conducted in accordance with principles such as those expressed in the Declaration of Helsinki (23) or equivalent statements prepared for use by national and/or multinational authorities (4).

67. For existing studies, both current standards and the standards pertaining at the time the study was performed should be taken into account.

68. The results of ethically and scientifically acceptable tests involving humans may be used, dependent on the regulatory position regarding the use of such data in an OECD member country, to derive reference values, including ARfDs, particularly in situations in which lower reference values would be derived when using these data.

69. The use of data from existing scientifically valid studies that are not compliant with ethical principles may be used, dependent on the regulatory position regarding the use of such data in an OECD member country, in the protection of human health if the findings indicate that human risk would be underestimated without the use of these findings.

70. If an acceptable risk assessment based on animal data cannot be achieved, alternative sources of information including mode of action should be considered. This information could be used to support a modification to the default safety factor applied to the PoD in an animal study according to the IPCS guidelines on setting Chemical Specific Assessment Factors (22). One alternative approach, might be to allow the use of data from scientifically valid human studies in setting reference values where the study was observational rather than experimental in design, or where the study investigated ADME at low levels of exposure in humans, and the results enabled the derivation of a chemical-specific adjustment factor (24). For example,

i. If the critical effect is mediated *via* receptor binding then *in vitro* work using human and animal derived material could be used to determine relative receptor binding affinities;

ii. If the critical effects are mediated *via* a metabolite, the relative rates and amounts of metabolite production can be determined in animal and human *in vitro* systems;

iii. Existing human data on the active substance or related molecules could be used to build a case (25);

iv. If sufficient information is available, a PBPK assessment could be performed.

H. Consideration of Different Subpopulations

71. It is important that the ARfD is adequate to protect the whole population (e.g., general, prenatal, postnatal, and older child).

72. The single exposure study in Annex 2 is based on testing in adult animals and thus intended to provide a health base value for the general population.

73. However, it is also important to ensure that the ARfD is adequate to protect the embryo/foetus from possible *in utero* effects. Therefore, use of data from developmental studies for the derivation of ARVs is considered, as a more conservative approach. Because of critical windows of sensitivity for developmental effects, it should be assumed that most developmental endpoints from repeat dose studies are relevant for setting acute dietary doses, unless there is evidence to the contrary (1) (2). There are several OECD Test Guidelines that serve to evaluate potential developmental toxicants following prenatal and postnatal exposures, including prenatal developmental toxicity (OECD TG414), reproductive (e.g., OECD TG416) and developmental neurotoxicity (OECD TG426) studies.

74. While an ARfD based on developmental (embryo/foetal) effects would be appropriate for women of child-bearing age, it is recognised that the same value may be overly conservative with respect to other subgroups in the population. Depending on the type of effect seen and the species evaluated, the use of an ARfD based on a developmental effect, e.g., skeletal or soft tissue malformations, could be inappropriate for children aged 1 to 6 years; as they are unlikely to be at risk for the developmental toxicity observed. In this situation, separate modelling with respect to acute dietary intake of residues can be performed taking into account age-specific acute consumption data. Alternatively, it might be necessary to address higher sensitivity of children to other forms of acute toxicity by testing during early life-stages.

75. Therefore, in some situations it may be necessary to set an ARfD for the general population and another value for other populations of concern.

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ANNEX 1

REFINEMENT OF THE EXPOSURE CALCULATION FOR THE ACUTE RISK ASSESSMENT

1. Acute exposure calculation and risk assessment (IESTI equation) currently follows the recommendations by the JMPR as laid down in the FAO manual on the submission and evaluation of pesticide residues data for the estimation of MRLs in food and feed (26) or equivalent national approaches (NESTI).

2. The JMPR had recently discussed the uncertainties in the calculation and interpretation of international estimated short-term intake (IESTI) (27) (28). In characterizing the risks associated with the short-term dietary exposure to a pesticide from the consumption of a certain food, the IESTI is compared with the established ARfD of the compound, and the intake expressed as a percentage of the ARfD. This value can then be used to make a judgment about the potential risk associated with the consumption of that food commodity. In a case where an IESTI calculation, for a crop/pesticide combination, results in an intake higher than 100% ARfD, the JMPR will state according to current practice: "The information provided to the JMPR precludes an estimate that the short-term dietary intake would be below the ARfD for the consumption of the commodity". On cases where the IESTI calculation results in an uptake greater than 100% of the ARfD, uncertainty analysis requires both the ARfD and exposure assessment to be revisited. Due to the overall uncertainties in the risk assessment, arising from the uncertainties in each of the parameters or assumptions used, an exceedance of the ARfD does not necessarily represent a health risk to the consumers. The establishment of an ARfD which is necessarily conservative and/or a conservative assessment of exposure will lead to an overly conservative estimate of acute dietary risk. However, if the acute risk assessment is too conservative, eventually it will not be viewed as credible.

3. Some governments, regional authorities, the CCPR and the JMPR have discussed the possibilities for improvement in the methodology currently used by the JMPR in assessing the short term dietary intake of pesticide residues. In this context, the 2007 JMPR Meeting also welcomed the publication of an Opinion by the European Food Safety Authority (EFSA) on 'Acute dietary intake assessment of pesticide residues in fruit and vegetables' (29).

4. Further approaches are under discussion but are not yet implemented.

5. Calculations of intake recognize four different cases (1, 2a, 2b and 3 below). Case 1 is the simple case where the residue in a composite sample reflects the residue level in a meal-sized portion of the commodity. Case 2 is the situation where the meal-sized portion as a single fruit or vegetable unit might have a higher residue than the composite. Case 2 is further divided into case 2a and case 2b where the unit size is less than or greater than the large portion size respectively. Case 3 allows for the likely bulking and blending of processed commodities such as flour, vegetable oils and fruit juices.

6. The following abbreviations are used in the equations:

LP:	Highest large portion reported (97.5th percentile of eaters)
HR:	Highest residue in composite sample of edible portion found in the supervised trials used for estimating the maximum residue level
bw:	Mean body weight
U:	Unit weight of the edible portion
v:	Variability factor - the factor applied to the composite residue to estimate the residue level in a high-residue unit
STMR:	Supervised trials median residue
STMR-P:	Supervised trials median residue in processed commodity

Case 1

7. The residue in a composite sample (raw or processed) reflects the residue level in a meal-sized portion of the commodity (unit weight is below 0.025 kg).

$$\text{IESTI} = \frac{\text{LP} \times (\text{HR})}{\text{bw}}$$

Case 2

8. The meal-sized portion, such as a single fruit or vegetable unit might have a higher residue than the composite (whole fruit or vegetable unit weight is above 0.025 kg).

Case 2a: Unit edible weight of raw commodity is less than large portion weight

$$\text{IESTI} = \frac{\text{U} \times (\text{HR}) \times v + (\text{LP-U}) \times (\text{HR})}{\text{bw}}$$

9. The Case 2a formula is based on the assumption that the first unit contains residues at the [HR × *v*] level and the next ones contain residues at the HR level, which represents the residue in the composite from the same lot as the first one.

Case 2b: Unit edible weight of raw commodity exceeds large portion weight

$$\text{IESTI} = \frac{\text{LP} \times (\text{HR}) \times v}{\text{bw}}$$

10. The Case 2b formula is based on the assumption that there is only one consumed unit and it contains residues at the [HR × *v*] level.

Case 3

11. Case 3 is for those processed commodities where bulking or blending means that the STMR-P represents the likely highest residue.

$$IESTI = \frac{LP \times STMR\text{-}P}{bw}$$

12. It should be noted, that an HR for the edible portion cannot always be derived, because only data on the whole commodity are available. Then the first step acute exposure calculation would be based on the highest residue in the whole raw agricultural commodity (RAC). First refinement option here would be to generate supervised trials residue data referring to the edible portion (e.g., citrus fruit, banana, kiwi fruit or pineapple without peel or mango, peach without stone) and to derive an HR from those trials.

13. Acute exposure calculations based on the HR might still result in an exceedance of an ARfD and require further exposure refinement.

14. The HR is usually derived from supervised field trials that have been conducted according to the maximum Good Agricultural Practice (GAP). It is based on the edible part of the raw commodity in most cases. However, some RACs are always processed before consumption by the public (e.g., potatoes, sugar beet, rape seed). The refined dietary exposure assessment refers to "food as eaten" and takes into account processing factors and residues in the edible portion as appropriate. HR values in the equations are replaced by the corresponding HR-P values (with "P" being the processing factor). More guidance on processing studies and processing factors can be found in OECD TG508 "Magnitude of Pesticide Residues in Processed Commodities".

15. Another refinement option is the more detailed analysis of consumption data and the refinement of LP. In many consumption surveys individual intakes of commodities arising from various food items are aggregated over the day based on the RAC. Due to this combination, information about the processing state of the food is lost: e.g., the intake of raw apples, apple juice and apple pie are combined to a total figure for apples based on the RAC. This aggregation normally results in an overestimation of the exposure and should be taken into account, if further information is available. Another important factor is the selection of the appropriate subgroup for the dietary risk assessment.

16. A further refinement option is the replacement of the default variability factor *v* by experimental data. Though on FAO/WHO level a default factor of 3 is already used, which cannot be reduced much further by using experimental data, EU Member States on the other hand still use factors of 5, 7 and 10, depending on the commodity. In those cases it might be appropriate to conduct a supervised residue study to determine the unit to unit variability. Data should be representative for different fruit sizes and fruit exposure situations. For statistical reasons, at least a total of 120 single units should be analyzed. According to Hamilton et al. (30) at least 119 samples are needed to estimate the 97.5 percentile with a 95 % confidence interval.

17. It was concluded by the JMPR (27) (28) that the IESTI and the ARfD values are not absolute numbers but are associated with uncertainty and variability. While it is possible to reduce uncertainty, biological variability can only be characterized. Both are set conservatively and the degree of conservatism reflects the level of uncertainty and variability in the data. The IESTI calculation should assist the decision making process rather than be the sole determinant of acceptable or unacceptable risk. The calculation takes into account only the parameters presented to it. In order to improve the estimation process the uncertainty of the individual components of the estimation should be examined and possible ways of improvements be identified.

18. It is recommended that the main objectives in the exposure refinement would be the improvement of the estimation of the short-term dietary intake of pesticides and that the refinement should include *inter alia* the following specific issues:

- Uncertainty and variability of the parameters used in the estimation;

- Ways to improve the consumption, unit weight and body weight data provided to the JMPR;
- Identification of additional subgroups of the population for which the assessment should be conducted, e.g., toddlers;
- The adequacy of the IESTI equations when residues from monitoring/enforcement data are used or the need of a specific methodology for this application;

ANNEX 2

GUIDANCE FOR CONDUCTING A SINGLE EXPOSURE TOXICITY STUDY

1. This is not a Test Guideline, only an advice, how to perform and tailor a single exposure test, what are the minimum parameters, depending on all available data.

INITIAL CONSIDERATIONS

2. In 2002 an analysis of the ARfD values set by several regulatory bodies was performed (1). There were large differences in the ARfD values between the analysed regulatory bodies (up to 2500-fold for some individual pesticides). As a result of this analysis it was concluded that "the current database of toxicological studies is not optimal for the derivation of the ARfD. More specific information on the acute toxicity other than lethality is often needed for setting an adequate ARfD." In the mean time the regulatory authorities made more comprehensive experiences with the derivation of ARfD values and notifiers and authorities made also the first experiences with the design of additional acute or short term studies for the derivation of ARfDs. Therefore, it was considered necessary to perform a new analysis in order to identify the toxicological studies on which the ARfD values have been based as of 2008. This analysis was recommended as a basis for a harmonized guidance on how to use available data on ARfD derivation and also for the development of an ARfD study design. The current analysis of the ARfD values was based on the last revision of this annotated list of active pesticide substances, published by EFSA on its website in November 2008 (31). The data basis for the ARfD derivation of 198 substances was analysed. The portion relying on special ARfD studies is very low; only 4% of the ARfDs are based on such studies. In some cases such special ARfD studies have not been accepted by the authorities because of quality deficiencies as a result of several criteria including absence of a guidance document. Therefore, in the EU peer review process some of the submitted special ARfD studies have not been used for the ARfD derivation.

3. These findings indicate that the development of an acute study design that produces more comprehensive toxicological data for setting ARfDs would be of value for avoiding poorly designed studies.

4. This *in vivo* single exposure study is not intended to become a routine data requirement. As discussed in the guidance document, the single exposure study should refine the ARfD and only be considered after the available toxicology and exposure information a compound has been appropriately evaluated. The relevant species and toxicological endpoints should already be documented and reasonably well understood because this study is only designed to refine endpoints and doses of concern in the existing repeat dose studies. Observations on the experimental animals are based on those listed in the OECD TG407.

PRINCIPLE OF THE TEST

5. An important principle in the design of the single exposure study is to consider all available information on the substance (e.g., physico-chemical, toxicokinetic and toxicodynamic properties of the test substance, available relevant information on structural analogues of the substance, results of previously conducted toxicity studies of the test substance) so that this study is conducted in the most appropriate way.

6. Some information on ADME may be able to be derived from chemical structure and physico-chemical data and results from toxicity studies (e.g., on NOAEL, indications of induction of metabolism). The collection of all available information is important for a decision on the route of administration, the choice of the vehicle, the selection of animal species, and the selection of dose levels and possibly for modifications of the dosing schedule.

7. The test substance is administered orally as a single exposure in graduated dose levels to several groups of animals, one dose being used per group. A vehicle control group is also included. Adherence to the specifications of OECD Guidance Document No.19 is stressed (19). Based on the definition of the ARfD, the acute intake is generally assessed on a per day basis. A worst-case exposure scenario would be to assume that daily intake occurs in a single meal.

8. For animal welfare reasons, the single exposure study protocol is not intended to examine reversibility of acute effects. Although reversibility can be one of the key criteria in arriving at a judgment on the adversity of an effect and the inclusion of recovery periods may be also helpful for the assessment of risk from intermittent exposures, this information should only be considered on the available data from repeat dose studies, since specific testing of reversibility would require more animals and this should be avoided.

9. The objective of the single exposure study is NOT

- To identify lethal doses or provide data on mortality after acute exposure to a chemical,
- To investigate the reversibility of acute effects,
- To investigate developmental effects, or
- To investigate corrosive/irritation properties.

DESCRIPTION OF THE METHOD

10. This protocol covers investigations of a comprehensive range of relevant endpoints which may arise after a single exposure, or during one day of dietary exposure to a test substance. In particular, it is tailored to determine the most appropriate PoD to derive a refined ARV. Special emphasis is placed on evaluating whether toxic effects observed in the standard package of repeat dose toxicity studies may also occur after single doses. It can also address additional parameters not usually examined in repeat dose studies, as well as provide further information on the dose-response curve and time to peak of acute toxic effects after a single exposure. The introduction of a new animal test for acute toxicity; such as the deleted OECD TG401 for lethality, is definitely not the goal of this project.

11. The HESI ACSA Committee designed an animal single exposure study to provide data relevant to 1-day human exposure with full evaluation at 24 hours and 7 days, to include histology, clinical chemistry, haematology and other specialized investigations that may be indicated by structure-activity or information from other studies as a first step of the proposed tiered approach (14). The HESI ACSA approach outlines also a draft protocol for a single exposure test in dogs or rodents.

12. This single exposure study should be performed only after determining that the oral route is the most likely exposure route or route of most concern to humans.

Selection of Animal Species

13. The selection of animal species should be based on the results of the repeat dose studies, which usually restrict the choice to the rat or the dog. It should not be necessary to perform the study in both species. Occasionally, mice may be more sensitive than rats or a better model for humans. If the

mouse is the preferred rodent species, the principles described for the rat should be adapted accordingly. There are for example differences in the activity of enzymes in the tyrosine catabolic pathway between rats and humans. Toxic effects of some active substances in rats are largely attributable to increased plasma tyrosine levels following HPPD inhibition. Therefore, in these cases the mouse is more predictive of the exposure in humans. Rabbits are not recommended for such single exposure studies (see Section F).

14. A justification should be given for the selection of the species. It should be demonstrated that the animals selected will respond to the relevant parameters with a higher sensitivity than other species and/or to be more relevant to human health risk assessment. Preferably, the animals used in this study should be from the same strain and source as the animals used in the key studies of the existing toxicological database for the test substance.

Housing and Feeding Conditions

15. The feed should be analysed for contaminants. A sample of the diet should be retained until finalisation of the report.

Preparation of Animals

16. Healthy young adult animals are randomly assigned to the control and treatment groups. Cages should be arranged in such a way that possible effects due to cage placement are minimised. The animals are identified uniquely and kept in their cages for at least 5 days prior to the start of the study to allow for acclimatisation to the laboratory conditions.

Preparation of Doses

17. Where necessary, the test substance is dissolved or suspended in a suitable vehicle. The toxic characteristics of vehicles other than water should be known. The homogeneity of the test substance in the vehicle should be assured.

PROCEDURE

Study Duration

18. Animals should be terminated at 24 hours if the toxic effect of interest is expected to be manifested within this time period. A later time point could be included to pick up latent effects if it is anticipated that the toxicities of interest will not be adequately evaluated by 24 hours. Selection of appropriate time points should be guided by existing knowledge. Appropriate justification should be submitted to explain the inclusion or exclusion of a second time point.

Number and Sex of Animals

Numbers of Animals

19. For reasons of animal welfare the numbers of animals used should be minimized without compromising the ability to characterize the effects and determine a robust PoD. This could be informed by statistical power calculations and the variability of the specific endpoints noted in the repeat dose studies as being especially relevant. Group sizes can be found in toxicology Test Guidelines (OECD TG407 for rodents and TG409 for dogs). For each dose, equal numbers of animals should be sacrificed at termination (e.g., 24 hours). If in exceptional cases where a second time point is justified and serial measurement cannot be performed on the same animals, the additional subgroup should be of the same size.

20. If a vehicle is used, a negative control is not required in addition to a vehicle control. If only one sex is evaluated, then the number of animals could be increased, if necessary, to provide more power to detect the toxicity of interest or more dose-groups could be included to provide data for benchmark modelling.

21. If identification of the toxic effect(s) of interest is possible in live animals at the 24 hour time point, an additional subgroup may not be necessary, as it may be sufficient to use the same group of animals for the sacrifice at the later time point for pathomorphological examinations.

Sex

22. Existing information should be used to tailor and appropriately focus this study. If existing data on the chemical show that one sex is clearly and consistently much more sensitive than the other for the endpoint(s) identified as being relevant for acute toxicity, then the study design should only use the more sensitive sex. Both males and females should only be used if necessary and justified.

Dose Selection

23. Normally three dose levels and a concurrent vehicle control should be used. Separate sets of animals should be used for each dose level. Dose levels should be selected taking into account any existing toxicity, ADME data available for the test compound and also information of likely human exposure. The data should be sufficient to produce a dose-effect curve. Thus, dose levels should be spaced to produce a gradation of toxic effects, ranging from recognisable toxicity but not death or severe suffering at the highest dose to no or only very slight effects at the low dose. If it is intended to establish a benchmark dose level rather than a PoD, it may be sensible to increase the number of dose groups. Use of a larger number of dose levels with a reduced spacing between doses may allow the study to be conducted with fewer animals per subgroup, depending on the statistical requirements for this approach.

24. The highest/overall PoD from the repeat dose studies using the same animal species could be selected as the low dose and together with one or two of the effect doses from the repeat dose studies. Special consideration should be given if the PoD from the repeat dose studies is representative for provoking acute effects, e.g., clinical effects observed at the beginning of a repeat dose study. If the test substance is a pesticide and the results of the study will be used for the derivation of an ARfD related to acute intake estimations, the high dose need not be greater than 500 mg/kg bw/d, which is the limit dose for setting an ARfD.

Administration of Doses

25. The most appropriate dosing would be by gavage in rodents and by capsule in dogs. Gavage should be done in a single dose to fasted animals using a stomach tube or a suitable intubation cannula. This dosing regimen would be particularly relevant when effects are C_{max}-dependent and rapidly reversible (e.g., inhibition of acetylcholinesterase by carbamates). However, other means of dosing may also be appropriate, but should be justified.

26. The maximum volume of liquid that can be administered at one time depends upon the size of the test animal. The volume should not exceed 1 mL/100 g bw, except for aqueous solutions, where 2 mL/100 g bw may be used. With the exception of irritating or corrosive substances, which are likely to cause exacerbated effects with higher concentrations, variability in volume should be minimised by adjusting the concentration to ensure a constant dosing volume at all dose levels.

27. Apart from treatment with vehicle instead of the test substance, the animals in the control group should be handled in an identical manner to those in the test group. If a vehicle is used to administer the test substance, the control group should receive the vehicle in the same volume used as total application volume (vehicle + test compound) in the treated groups. If different volumes are administered to the different treatment groups, the control should receive the vehicle at the highest volume used.

28. If administration is via feed in the dog, the single dose should be consumed completely in one meal within approximately one hour; a confirmation of this consumption time and achieved dose level should be provided in the study report. Data on the palatability of the intended dose levels in diet should be available.

Clinical Observations

29. Clinical observations should be made in all animals at least once before exposure to the test substance (to allow for within-subject comparisons) and at least 0.5, 1, 2, 4 and 24 hours after dosing. The peak period of the anticipated effects should be considered when determining the time points for clinical observations. If later time points are evaluated, further observations should be made at least twice daily after the first 24 hours.

30. Observations should be carefully recorded, preferably using scoring systems, explicitly defined/reported by the testing laboratory. Effort should be made to ensure that variations in the test conditions are minimal and that observer bias is excluded. Signs noted should include, but not be limited to, changes in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions and autonomic activity (e.g., lacrimation, piloerection, pupil size, and unusual respiratory pattern).

31. Changes in gait, posture, response to handling as well as the presence of clonic or tonic movements, stereotypy (e.g., excessive grooming, repetitive circling) or bizarre behaviour (e.g., self-mutilation, walking backwards) should also be recorded (see also Section E).

Body Weight and Food/Water Consumption

32. All animals should be weighed on the day of treatment and prior to sacrifice of the subgroup. If a later time point is included, the animals should be weighed every 24 hours after treatment. Measurements of food consumption and drinking water intake should be made daily.

Toxicokinetics

33. Available information on toxicokinetics should be considered before commencing this single exposure study. Frequently, toxicokinetic data will only be available for the rat, but not for the dog. If

collection of samples for substance plasma levels at different time points is considered to be useful, it can be incorporated into the design of the study provided that it does not interfere with other investigations. Blood samples should be taken at least at subgroup termination time points.

Functional Observations

34. If existing data indicate that the critical effect of the compound is neurotoxicity, then the acute neurotoxicity test guideline should be considered (see OECD TG424 and OPPTS 870.6200). Alternatively, the elements described in this guideline may be combined with the design of an acute neurotoxicity battery study, as long as none of the requirements of both guidelines are violated by the combination. The parameters included may be tailored based on the extent of existing knowledge.

35. If the test species used is the rat, sensory reactivity to stimuli of different types (e.g., auditory, visual, and proprioceptive stimuli), grip strength and motor activity should be assessed. This evaluation should be conducted in the peak period of the anticipated effect, e.g., 1, 2 or 4 hours, as well as just before sacrifice of the subgroups. If the peak effect is expected to be close to 24 hours, then the 24 hour observation is sufficient.

Haematology

36. The haematologic examination is only required if data from repeat dose studies indicate that the blood cells and/or the haematopoietic system are target sites. The following haematological examinations should be made just prior to or as part of the procedure for killing the animals at the end of the test period: haematocrit, haemoglobin concentration, erythrocyte count, total and differential leukocyte count, platelet count, and blood clotting time/potential. Justification should be given, if these parameters are not investigated.

Clinical Biochemistry

37. The clinical biochemistry examination is only required if data from repeat dose studies indicate that these parameters are of concern. The parameters evaluated may depend on the species selected (typically rat or dog) and on the results of the repeat dose studies. Clinical biochemistry determinations should be performed on blood samples of all animals taken just prior to or as part of the procedure for killing the animals in each subgroup at the end of the test period. In general, the following investigations of plasma or serum should be included: glucose, total cholesterol, urea, creatinine, total protein, albumin, at least two enzymes indicative of hepatocellular effects (e.g., alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma glutamyl transpeptidase, and sorbitol dehydrogenase). Measurements of additional enzymes and bile acids may provide useful information under certain circumstances.

38. In addition, the investigation of serum markers of acute tissue damage should be considered. These need to be identified for chemicals in certain classes or on a case-by-case basis. If a specific, potentially acute effect of the test substance has been observed using special techniques in repeat dose studies, then these techniques should also be used in this study:

- Cholinesterase inhibition in plasma, red blood cells, brain and peripheral nervous tissue should be measured for compounds known to inhibit these enzymes.

- Blood methaemoglobin should be measured for compounds known to increase methaemoglobin formation. In this case it is advisable that blood samples are obtained at the time of peak effect if it does not interfere with other investigations since Met-Hb formation is an acute effect and Met-Hb is rapidly degraded.

- For endocrine modulators, specific hormones, which could be affected after single exposure, should be measured[1].

Urinalysis

39. Urinalysis determinations are optional and only necessary if data from repeat dose studies indicate that this is a critical parameter to be evaluated. Urinalysis determinations should be performed just prior to termination. The following parameters should be evaluated: appearance, volume, osmolality or specific gravity, pH, protein, glucose, blood and blood cells, and cell debris.

Pathology

40. Methods for humane killing according to OECD Guidance Document No. 19 should be considered (19). The pathological and organ weight evaluations should focus on tissues/endpoints that are found to be targets in the repeat dose studies.

Gross necropsy

41. All animals in the study shall be subjected to a full, detailed gross necropsy which includes careful examination of the external surface of the body, all orifices, the cranial, thoracic and abdominal cavities and their contents.

42. The following tissues should be preserved in the most appropriate fixation medium for both the type of tissue and the intended subsequent histopathological examination: all gross lesions, brain (representative regions including cerebrum, cerebellum, and pons), spinal cord, stomach, small and large intestines (including Peyer's patches), liver, kidneys, adrenals, spleen, heart, thymus, thyroid, trachea, and lungs (preserved by inflation with fixative and then immersion), gonads, accessory sex organs (e.g., uterus, prostate), urinary bladder, lymph nodes (preferably one lymph node covering the route of administration and another one distant from the route of administration to cover systemic effects), peripheral nerve (sciatic or tibial) preferably in close proximity to the muscle, and a section of bone marrow (or, alternatively, a freshly mounted bone marrow aspirate). Specific attention should be paid to likely target organs based on the known properties of the test substance.

Organ weight

43. The following organs should be trimmed of any adherent tissue, as appropriate, and their wet weight should be measured as soon as possible after dissection to avoid drying: liver, kidneys, adrenals, testes, epididymides, thymus, and spleen.

44. In addition, if relevant as target organ for acute effects of the test substance, the wet weight should be determined for the following organs as soon as possible after dissection to avoid drying: paired ovaries, uterus, seminal vesicles (including coagulating glands), and prostate (dorsolateral and ventral part combined). Alternatively, seminal vesicles and prostate may be trimmed after fixation. Clamp or ligature should be present during fixation as leakage of fluid provokes damage to fine structures in seminal vesicles.

45. The following organs should be weighed after fixation: thyroid (trimming should also be performed after fixation in order to avoid tissue damage) and dorsolateral and ventral parts of the prostate separately after separation.

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[1] (OECD Test Guideline No. 407 Repeated Dose 28-Day Oral Toxicity Study in Rodents): The following factors may influence the variability and the absolute concentrations of the (thyroid) hormone:

- time of sacrifice because of diurnal variation of hormone concentrations
- method of sacrifice to avoid undue stress to the animals that may affect hormone concentrations
- test kits for hormone determinations that may differ by their standard curves.

Histopathology

46. Full histopathology should be carried out on the preserved organs and tissues of all animals in the control and high dose groups unless existing data from repeat dose studies indicate that an organ is not a target site. These examinations should be extended to animals of all other dose groups, if treatment-related changes are observed in the high dose group. All gross lesions shall be examined.

DATA AND REPORTING

47. Individual animal data should be provided. Additionally, all data should be summarised in tabular form showing, for each test group, the number of animals at the start of the test, the number of animals found dead during the test or sacrificed for humane reasons and their respective cause of death, the number showing signs of toxicity, a description of the signs of toxicity observed, including time of onset, duration, and severity, the number of animals showing lesions, the type of lesions and the percentage of animals displaying each type of lesion.

48. When possible, numerical results should be evaluated by an appropriate and generally acceptable statistical method. The statistical method should be selected during the design of the study.

Test Report

49. The test report should include the following information:

Aim of the study:

- Justification for conducting such a single exposure study
- Rationale for the specific design (e.g., choice of species and sex, dose selection, endpoint selection)

Guidelines and Quality Assurance:

- Test type (Guideline)
- GLP

Test substance:

- physical nature, purity, and physicochemical properties
- identification data

Test animals:

- species and strain used and justification for the selection of species and strain
- number, age, and sex of animals
- source, housing conditions, diet, etc.
- individual weight of animals at the start of the test

Test conditions:

- rationale for dose level selection
- details of test substance formulation/diet preparation, achieved concentration, stability, and homogeneity of the preparation
- details of the administration of the test substance
- conversion from diet test substance concentration (ppm) to the actual dose (mg/kg bw/d), if the test substance was administered via the diet

- details of food and water quality

Results:

- body weight/body weight changes
- food consumption, and water consumption, if applicable
- toxic response data by sex and dose level, including signs of toxicity
- nature, severity and duration of clinical signs
- functional observations (e.g., sensory reactivity, grip strength, motor activity assessments)
- haematological tests with relevant base-line values
- clinical biochemistry tests with relevant base-line values
- body weight at sacrifice and organ weight data
- gross necropsy findings
- a detailed description and tabulation of all histopathological findings
- statistical treatment of results

Summary and discussion of results

Conclusions, Critical effects, and PoD (or benchmark dose, if applicable)

ANNEX 3

LIST OF ACRONYMS

ACSA	Agricultural Chemical Safety Assessment
ADI	Acceptable Daily Intake
ADME	Absorption, Distribution, Metabolisation, and Excretion
ARfD	Acute Reference Dose
ARV	Acute Reference Value
BMD	Benchmark Dose
BMDL	Benchmark Dose Limit
CNS	Central Nerve System
CSAF	Chemical-Specific Adjustment Factor
EU	European Union
EEC	European Economic Community
FOB	Functional Observation Batteries
GI	Gastrointestinal
HESI	Health and Environmental Sciences Institute
ILSI	International Life Sciences Institute
IPCS	International Programme on Chemical Safety
JMPR	Joint Meeting on Pesticide Residues
MLD	Median Lethal Dose
MoE	Margin of Exposure
MRL	Maximum Residue Level
NOAEL	No Observed Adverse Effect Level
OECD	Organisation for Economic Co-operation and Development
PBPK	Physiologically-based Pharmacokinetic
PoD	Point of Departure
QSAR	Quantitative Structure-Activity Relationship
TDI	Tolerable Daily Intake
TG	Test Guideline
WHO	World Health Organization
WNT	Working Group of National Co-ordinators to the Test Guidelines Program